

**What is claimed is:**

1. A method of detecting an analyte, comprising:
  - (i) combining:
    - (a) an analyte;
    - (b) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a first oligonucleotide moiety comprising a first portion;
    - (c) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide;
    - (d) a hybridization blocker oligonucleotide, where the hybridization blocker oligonucleotide comprises a portion that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety;
  - (ii) forming a hybrid comprising the first portion of the first oligonucleotide moiety and the portion of the second oligonucleotide moiety, where the hybrid comprises a 3' terminus of the first or second oligonucleotide moieties;
  - (iii) extending the 3' terminus to produce an amplicon;
  - (iv) amplifying the amplicon to produce an amplification product; and
  - (v) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

2. The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' sequence that is not complementary to the first oligonucleotide moiety.

3. The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 5' sequence that is not complementary to the first oligonucleotide moiety.
4. The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' sequence and a 5' sequence that are not complementary to the first oligonucleotide moiety.
5. The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' cap that prevents 3' extension of the first oligonucleotide moiety by a DNA polymerase.
6. The method of claim 1, wherein the hybridization blocker oligonucleotide forms a hybrid comprising all of the bases of the first portion of the first oligonucleotide moiety.
7. The method of claim 6, wherein the first portion of the first oligonucleotide moiety is about 10 bases in length and the hybridization blocker oligonucleotide is about 18 bases in length.
8. The method of claim 6, wherein the first portion of the first oligonucleotide moiety is about the length of the entire first oligonucleotide moiety.
9. The method of claim 1, wherein the hybridization blocker oligonucleotide forms a hybrid comprising less than all of the bases of the first portion of the first oligonucleotide moiety.
10. The method of claim 1, further comprising combining a deblocker oligonucleotide that is capable of reducing the presence of a hybrid between the hybridization blocker oligonucleotide and the first oligonucleotide.
11. The method of claim 10, wherein the deblocker oligonucleotide comprises a first portion that is capable of forming a hybrid with the portion of the hybridization blocker oligonucleotide that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.

12. The method of claim 11, wherein the deblocker oligonucleotide comprises a second portion that is capable of forming a hybrid with a portion of the hybridization blocker oligonucleotide that does not form a hybrid with the first oligonucleotide moiety.
13. The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a double-stranded portion that is 3' of the portion of the hybridization blocker that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.
14. The method of claim 13, wherein the double-stranded portion comprises a hairpin loop.
15. The method of claim 10, wherein the hybridization blocker oligonucleotide is combined before the deblocker oligonucleotide.
16. The method of claim 10, wherein the hybridization blocker oligonucleotide is combined after the deblocker oligonucleotide.
17. The method of claim 10, wherein the hybridization blocker oligonucleotide is combined simultaneously with the deblocker oligonucleotide.
18. The method of claim 1, wherein the hybridization blocker oligonucleotide is combined before the analyte and first and second proximity members.
19. The method of claim 1, wherein the hybridization blocker oligonucleotide is combined after the analyte and first and second proximity members.
20. The method of claim 1, further comprising combining a second hybridization blocker oligonucleotide that is capable of hybridizing to the portion of the second oligonucleotide moiety that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.
21. The method of claim 1, wherein said amplifying is by a method selected from the group consisting of polymerase chain reaction, strand displacement amplification, thermophilic strand displacement amplification, self-sustained sequence replication, nucleic acid sequence-based

amplification, a Q $\beta$  replicase system, ligase chain reaction, and transcription mediated amplification.

22. The method of claim 1, wherein the blocker reduces analyte-independent formation of the amplicon by a factor of at least 100-fold.

23. The method of claim 22, wherein the blocker reduces analyte-independent formation of the amplicon by a factor of at least 1000-fold.

24. The method of claim 1, wherein the analyte is capable of being detected at a concentration of at least about 1 pM.

25. The method of claim 24, wherein the analyte is capable of being detected at a concentration of at least about 0.1 pM.

26. The method of claim 25, wherein the analyte is capable of being detected at a concentration of at least about 0.01 pM.

27. The method of claim 1, wherein the detecting is quantitative.

28. The method of claim 1, wherein the first or second analyte-specific binding entity is a protein complex.

29. The method of claim 28, wherein the protein complex comprises a first protein that is conjugated to the oligonucleotide moiety and a second protein that is capable of forming a complex with the analyte.

30. The method of claim 29, wherein the first protein is selected from the group consisting of Protein A and Protein G.

31. A method of detecting an analyte, comprising:

(i) combining:

(a) an analyte;

- (b) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a first oligonucleotide moiety comprising a first portion and a second portion;
- (c) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide;
- (d) a support comprising a capture oligonucleotide that is capable of hybridizing to the second portion of the first oligonucleotide moiety;
- (ii) forming a hybrid comprising the first portion of the first oligonucleotide moiety and the portion of the second oligonucleotide moiety;
- (iii) extending the 3' terminus to produce an amplicon;
- (iv) amplifying the amplicon to produce an amplification product; and
- (v) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

32. The method of claim 31, wherein the first and second portions of the first oligonucleotide comprise no overlapping bases.

33. The method of claim 31, wherein the first and second portions of the first oligonucleotide comprise contiguous bases in common.

34. The method of claim 31, wherein the first and second portions of the first oligonucleotide are the same base sequence.

35. The method of claim 31, wherein the hybrid comprises a 3' terminus of the first or second oligonucleotide moieties and said producing the amplicon comprises extending the 3' terminus.
36. The method of claim 31, wherein said producing the amplicon comprises a ligation step.
37. The method of claim 31, comprising forming a hybrid between the capture oligonucleotide and the first oligonucleotide moiety after said combining.
38. The method of claim 37, further comprising washing the support after said forming a hybrid between the capture oligonucleotide and the first oligonucleotide moiety.
39. The method of claim 38, further comprising releasing the first proximity member from the support after said washing the support.
40. The method of claim 39, wherein the hybrid between the capture oligonucleotide and the first oligonucleotide moiety comprises a restriction endonuclease recognition site, and wherein said releasing comprises cleaving the site by a cognate restriction endonuclease.
41. The method of claim 39, further comprising a polymerase-catalyzed extension of the hybrid between the capture oligonucleotide and the first oligonucleotide moiety to form a restriction endonuclease recognition site, wherein said releasing comprises cleaving the site by a cognate restriction endonuclease.
42. The method of claim 39, wherein the releasing is by physical dissociation of the hybrid between the capture oligonucleotide and the first oligonucleotide moiety.
43. The method of claim 39, wherein the releasing is by physical, chemical or enzymatic cleavage.
44. The method of claim 39, wherein the releasing comprises extending a double-stranded portion of the capture oligonucleotide to displace the first oligonucleotide moiety from the hybrid between the capture oligonucleotide and the first oligonucleotide moiety.

45. The method of claim 44, wherein the double-stranded portion of the capture oligonucleotide comprises a hairpin loop.
46. The method of claim 39, wherein the releasing comprises extending a double-stranded portion of the first oligonucleotide moiety to displace the capture oligonucleotide from the hybrid between the capture oligonucleotide and the first oligonucleotide moiety.
47. The method of 46, wherein the double-stranded portion comprises a hairpin loop.
48. The method of claim 39, wherein one or both strands of the hybrid between the capture oligonucleotide and the first oligonucleotide moiety comprises RNA, and wherein the releasing comprises degrading said one or both strands with a RNase.
49. The method of claim 31, wherein said amplifying is by a method selected from the group consisting of polymerase chain reaction, strand displacement amplification, thermophilic strand displacement amplification, self-sustained sequence replication, nucleic acid sequence-based amplification, a Q $\beta$  replicase system, ligase chain reaction, and transcription mediated amplification.
50. The method of claim 31, wherein the detecting is quantitative.
51. A method of detecting an analyte, comprising:
- (i) combining:
    - (a) an analyte;
    - (b) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a tether oligonucleotide moiety comprising a first portion and a second portion;

- (c) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to an oligonucleotide moiety comprising a first portion;
- (d) a splint oligonucleotide, comprising (i) a first portion that is capable of hybridizing to the first portion of the oligonucleotide moiety and (ii) a second portion that is capable of hybridizing to the first portion of the tether oligonucleotide moiety;
- (ii) forming:
  - (a) a first hybrid comprising the first portion of the oligonucleotide moiety, the first portion of the splint oligonucleotide, and an extendable terminus of either the oligonucleotide moiety or the splint oligonucleotide; and
  - (b) a second hybrid comprising the first portion of the tether oligonucleotide and the second portion of the tether oligonucleotide;
- (iii) extending the extendable terminus, thereby producing an amplicon;
- (iv) amplifying the amplicon to produce an amplification product; and
- (v) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

52. The method of claim 51, wherein the oligonucleotide moiety comprises the extendable terminus.

53. The method of claim 51, wherein the splint oligonucleotide comprises the extendable terminus.

54. The method of claim 51, wherein said extending the extendable terminus displaces the tether oligonucleotide from the second hybrid.



55. The method of claim 54, wherein the tether oligonucleotide is displaced by strand displacement.
56. The method of claim 54, wherein the tether oligonucleotide is displaced by hydrolysis catalyzed by a polymerase having a 3'-5' exonuclease activity.
57. The method of claim 51, wherein said amplifying is by a method selected from the group consisting of polymerase chain reaction, strand displacement amplification, thermophilic strand displacement amplification, self-sustained sequence replication, nucleic acid sequence-based amplification, a Q $\beta$  replicase system, ligase chain reaction, and transcription mediated amplification.
58. The method of claim 51, wherein the splint oligonucleotide further comprises a restriction endonuclease recognition site located 3' of the second portion and a first primer binding site located 3' of the restriction endonuclease recognition site and 5' of the first portion, and where the oligonucleotide moiety comprises a second primer binding site located 5' of the first portion of the oligonucleotide moiety.
59. The method of claim 58, wherein said amplifying is by strand displacement amplification comprising using first and second primers that hybridize to the first and second primer binding sites, respectively, and a restriction endonuclease that nicks its cognate recognition site.
60. The method of claim 51, wherein the detecting is quantitative.
61. The method of claim 51, wherein said producing the amplicon comprises a ligation step.
62. A method of detecting an analyte, comprising:
- (i) combining:
    - (a) an analyte;

- (b) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a first tether oligonucleotide moiety comprising a first portion;
- (c) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second tether oligonucleotide moiety comprising a first portion;
- (d) a first splint oligonucleotide, comprising (i) a first portion that is capable of hybridizing to the first portion of the first tether oligonucleotide moiety and (ii) a second portion that is capable of hybridizing to a second portion of a second splint oligonucleotide;
- (e) a second splint oligonucleotide, comprising (i) a first portion that is capable hybridizing to the first portion of the second tether oligonucleotide moiety and (ii) a second portion that is capable of hybridizing to the second portion of the first splint oligonucleotide;
- (ii) forming:
  - (a) a first hybrid comprising the first portion of the first tether oligonucleotide moiety and the first portion of the first splint oligonucleotide;
  - (b) a second hybrid comprising the first portion of the second tether oligonucleotide and the first portion of the second splint oligonucleotide;
 and

- (c) a third hybrid comprising the second portions of the first and second splint oligonucleotides, a 3' terminus of the first splint oligonucleotide, and a 3' terminus of the second splint oligonucleotide;
- (iii) extending the 3' termini of the third hybrid, thereby producing an amplicon;
- (iv) amplifying the amplicon to produce an amplification product; and
- (v) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

63. The method of claim 62, wherein a 3' terminus or a 5' terminus of the first tether oligonucleotide is conjugated to the first analyte-specific binding entity, and a 3' terminus of the second tether oligonucleotide is conjugated to the second analyte-specific binding entity.

64. The method of claim 62, wherein said producing an amplicon displaces the first and second tether oligonucleotides from the first and second hybrids.

65. The method of claim 62, wherein the first and second tether oligonucleotides are displaced by strand displacement.

66. The method of claim 62, wherein the first and second tether oligonucleotides are displaced by hydrolysis catalyzed by a polymerase having a 3'-5' exonuclease activity.

67. The method of claim 62, further comprising a wash step after said extending and before said amplifying that substantially removes the amplicon from the first and second proximity members.

68. The method of claim 62, wherein the detecting is quantitative.

69. The method of claim 62, wherein said producing the amplicon comprises a ligation step.

70. A method of detecting an analyte, comprising:

- (i) combining:

- (a) an analyte;
- (b) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a first oligonucleotide moiety comprising a restriction endonuclease recognition site, a 3' cap that prevents 3' extension of the first oligonucleotide moiety by a DNA polymerase, and a first portion that is 3' of the restriction endonuclease recognition site;
- (c) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a first portion that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety;
- (ii) forming a hybrid comprising the first portions of the first and second oligonucleotide moieties and a 3' terminus of the second oligonucleotide;
- (iii) extending the 3' terminus of the second oligonucleotide moiety, thereby making the restriction endonuclease recognition site of the first oligonucleotide moiety double-stranded;
- (iv) nicking the restriction endonuclease recognition site of the first oligonucleotide moiety;
- (v) extending the first oligonucleotide moiety from the nick to displace the downstream portion of the first oligonucleotide moiety;
- (vi) performing strand displacement amplification from the restriction endonuclease recognition site to produce an amplification product; and

(vii) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

71. The method of claim 70, wherein the second oligonucleotide moiety comprises a restriction endonuclease recognition site, and the strand displacement amplification is from the restriction endonuclease recognition sites of the first and second oligonucleotide moieties.

72. The method of claim 70, wherein the detecting is quantitative.

73. The method of claim 70, wherein a 5' terminus of the first oligonucleotide moiety is conjugated to the first analyte-specific binding entity, and a 5' terminus of the second oligonucleotide moiety is conjugated to the second analyte-specific binding entity.

74. A method of detecting an analyte, comprising:

(i) combining:

(a) an analyte;

(b) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a first oligonucleotide moiety comprising a first portion;

(c) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a first portion;

(ii) forming at least one hybrid comprising the first portion of the first oligonucleotide moiety and the first portion of the second oligonucleotide moiety, wherein the at least one hybrid comprises a 3' terminus that is capable of being extended to form a complement of a second portion of the first oligonucleotide moiety that comprises a 5' terminus;

- (iii) producing an amplicon;
- (iv) amplifying the amplicon to produce an amplification product; and
- (v) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

75. The method of claim 74, wherein the at least one hybrid comprises the first portions of the first and second oligonucleotides.

76. The method of claim 74, wherein two hybrids are formed, the first hybrid comprising the first portion of the first oligonucleotide moiety and a first portion of a splint oligonucleotide, and the second hybrid comprising the first portion of the second oligonucleotide moiety and a second portion of the splint oligonucleotide.

77. The method of claim 74, wherein said producing an amplicon comprises extending the 3' terminus.

78. The method of claim 74, wherein said producing an amplicon comprises: (i) forming a hybrid between the second portion of the first oligonucleotide moiety and a third oligonucleotide and (ii) ligating the 3' terminus of the at least one hybrid to a 5' terminus of the third oligonucleotide.

79. A method of detecting an analyte, comprising:

- (i) combining:
  - (a) an analyte comprising at least two antigen-specific binding sites;
  - (b) a first proximity member, comprising a first antigen that is conjugated to a first oligonucleotide moiety comprising a first portion, wherein the first antigen is capable of forming a complex with one of the antigen-binding sites of the analyte;

- (c) a second proximity member, comprising a second antigen that is conjugated to a second oligonucleotide moiety comprising a first portion, wherein the second antigen is capable of forming a complex with another antigen-binding site of the analyte;
- (ii) forming a hybrid comprising the first portions of the first and second oligonucleotide moieties to produce an amplicon;
- (iii) amplifying the amplicon to produce an amplification product; and
- (iv) detecting the amplification product;

wherein detection of the amplification product allows detection of the analyte.

80. The method of claim 79, wherein the detecting is quantitative.

81. The method of claim 79, wherein the analyte is an antigen-specific immunoglobulin.

82. The method of claim 79, wherein the hybrid comprises a 3' terminus of the first or second oligonucleotide moieties.

83. A kit, comprising:

- (a) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with an analyte and that is conjugated to a first oligonucleotide moiety comprising a first portion;
- (b) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide; and

- (c) a hybridization blocker oligonucleotide, wherein the hybridization blocker oligonucleotide comprises a portion that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.

84. The kit of claim 83, wherein the hybridization blocker oligonucleotide comprises a 3' sequence that is not complementary to the first oligonucleotide moiety.

85. The kit of claim 83, wherein the hybridization blocker oligonucleotide comprises a 5' sequence that is not complementary to the first oligonucleotide moiety.

86. The kit of claim 83, wherein the hybridization blocker oligonucleotide comprises a 3' sequence and a 5' sequence that are not complementary to the first oligonucleotide moiety.

87. The kit of claim 83, wherein the hybridization blocker oligonucleotide comprises a 3' cap that prevents 3' extension of the first oligonucleotide moiety by a DNA polymerase.

88. The kit of claim 83, wherein the hybridization blocker oligonucleotide is capable of forming a hybrid comprising all of the first portion of the first oligonucleotide moiety.

89. The kit of claim 83, wherein the hybridization blocker oligonucleotide is capable of forming a hybrid comprising less than all of the first portion of the first oligonucleotide moiety.

90. The kit of claim 83, further comprising a deblocker oligonucleotide that is capable of reducing the presence of a hybrid between the hybridization blocker oligonucleotide and the first oligonucleotide.

91. The kit of claim 90, wherein the deblocker oligonucleotide comprises a first portion that is capable of forming a hybrid with the portion of the hybridization blocker oligonucleotide that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.



92. The kit of claim 91, wherein the deblocker oligonucleotide comprises a second portion that is capable of forming a hybrid with a portion of the hybridization blocker oligonucleotide that does not form a hybrid with the first oligonucleotide moiety.

93. The kit of claim 92, wherein the hybridization blocker oligonucleotide comprises a double-stranded portion that is 3' of the portion of the hybridization blocker that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.

94. The kit of claim 93, wherein the double-stranded portion comprises a hairpin loop.

95. The kit of claim 83, further comprising a second hybridization blocker oligonucleotide that is capable of hybridizing to the portion of the second oligonucleotide moiety that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.

96. A kit, comprising:

- (a) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with an analyte and that is conjugated to a first oligonucleotide moiety comprising a first portion and a second portion;
- (b) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide; and
- (c) a support comprising a capture oligonucleotide that is capable of hybridizing to the second portion of the first oligonucleotide moiety.

97. The kit of claim 96, wherein the first and second portions of the first oligonucleotide comprise no overlapping bases.
98. The kit of claim 96, wherein the first and second portions of the first oligonucleotide comprise contiguous bases in common.
99. The kit of claim 96, wherein the first and second portions of the first oligonucleotide are the same base sequence.
100. The kit of claim 96, wherein the capture oligonucleotide or the first oligonucleotide moiety comprises one strand of a restriction endonuclease recognition site.
101. The kit of claim 96, wherein the capture oligonucleotide comprises a double-stranded portion.
102. The kit of claim 101, wherein the double-stranded portion of the capture oligonucleotide comprises a hairpin loop.
103. The kit of claim 96, wherein the capture oligonucleotide comprises an RNA portion that is capable of forming a hybrid with the first oligonucleotide moiety.
104. The kit of claim 96, wherein the first oligonucleotide moiety comprises an RNA portion that is capable of forming a hybrid with the capture oligonucleotide.
105. A kit, comprising:
- (a) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with an analyte and that is conjugated to a tether oligonucleotide moiety comprising a first portion;
  - (b) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to an oligonucleotide moiety comprising a first portion; and

- (c) a splint oligonucleotide, comprising (i) a first portion that is capable of hybridizing to the first portion of the oligonucleotide moiety and (ii) a second portion that is capable of hybridizing to the first portion of the tether oligonucleotide moiety.

106. The kit of claim 105, wherein a 3' terminus of the tether oligonucleotide is conjugated to the first analyte-specific binding entity, and a 5' terminus of the oligonucleotide moiety is conjugated to the second analyte-specific binding entity.

107. The kit of claim 105, wherein the splint oligonucleotide further comprises a restriction endonuclease recognition site located 3' of the second portion and a first primer binding site located 3' of the restriction endonuclease recognition site and 5' of the first portion, and wherein the oligonucleotide moiety comprises a second primer binding site located 5' of the first portion of the oligonucleotide moiety.

108. A kit, comprising:

- (a) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with an analyte and that is conjugated to a first tether oligonucleotide moiety comprising a first portion;
- (b) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second tether oligonucleotide moiety comprising a first portion;
- (c) a first splint oligonucleotide, comprising (i) a first portion that is capable of hybridizing to the first portion of the first tether oligonucleotide moiety

and (ii) a second portion that is capable of hybridizing to a second portion of a second splint oligonucleotide; and

- (d) a second splint oligonucleotide, comprising (i) a first portion that is capable hybridizing to the first portion of the second tether oligonucleotide moiety and (ii) a second portion that is capable of hybridizing to the second portion of the first splint oligonucleotide.

109. The kit of claim 108, wherein a 3' or 5' terminus of the first tether oligonucleotide is conjugated to the first analyte-specific binding entity, and a 3' terminus of the second tether oligonucleotide is conjugated to the second analyte-specific binding entity.

110. A kit, comprising:

- (a) a first proximity member, comprising a first analyte-specific binding entity that is capable of forming a complex with an analyte and that is conjugated to a first oligonucleotide moiety comprising a restriction endonuclease recognition site, a 3' cap that prevents 3' extension of the first oligonucleotide moiety by a DNA polymerase, and a first portion that is 3' of the restriction endonuclease recognition site; and
- (b) a second proximity member, comprising a second analyte-specific binding entity that is capable of forming a complex with the analyte and that is conjugated to a second oligonucleotide moiety comprising a first portion that is capable of forming a hybrid with the first portion of the first oligonucleotide moiety.

111. The kit of claim 110, wherein the second oligonucleotide moiety comprises a restriction endonuclease recognition site that is 5' of the first portion.

112. The kit of claim 110, wherein a 5' terminus of the first oligonucleotide moiety is conjugated to the first analyte-specific binding entity, and a 5' terminus of the second oligonucleotide moiety is conjugated to the second analyte-specific binding entity.

113. A kit, comprising:

- (a) a first proximity member, comprising a first antigen that is conjugated to a first oligonucleotide moiety comprising a first portion, wherein the first antigen is capable of forming a complex with an antigen-binding site of an analyte comprising at least two antigen-specific binding sites; and
- (b) a second proximity member, comprising a second antigen that is conjugated to a second oligonucleotide moiety comprising a first portion, where the second antigen is capable of forming a complex with another antigen-binding site of the analyte.

114. A method of quantifying a non-nucleic acid analyte, comprising:

- (i) forming a plurality of standard samples comprising (a) a first and second proximity member, each comprising an analyte-specific binding entity conjugated to an oligonucleotide moiety, (b) a known starting quantity of a nucleic acid control, and (c) a known quantity of a non-nucleic acid analyte, thereby forming amplicons comprising a portion of the first and second oligonucleotide moieties;
- (ii) forming at least one test sample comprising (a) a first and second proximity member, each comprising an analyte-specific binding entity conjugated to an oligonucleotide moiety, (b) the same known starting quantity of a nucleic acid control, and (c) an unknown quantity of the non-nucleic acid analyte, thereby

forming an amplicon comprising a portion of the first and second oligonucleotide moieties;

- (iii) amplifying the amplicons and the nucleic acid controls;
- (iv) measuring the amplified amplicons and nucleic acid controls to determine a measured indicia;
- (v) determining a calibration curve from the measured indicia of the plurality of standard samples; and
- (vi) comparing the measured indicia of the at least one test sample with the calibration curve to determine the quantity of the non-nucleic acid analyte in the test sample.